Myocardial Protective Effects of Nicardipine Hydrochloride (Perdipine) in Glucose Potassium Cardioplegic Solution on Isolated Rat Heart

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The effect on myocardial protection against ischemic damage by addition of the Ca antagonist perdipine (nicardipine) at 0.25mg/L, and 0.5mg/L, to cardioplegic solution on isolated rat heart was assessed by hemodynamic studies, the time required to achieve resuscitation, CPK assay, and histologic evaluation.

Twenty seven rat heart were divided into three groups of nine, with two experimental groups receiving different amounts of perdipine in the cardioplegic solution and group serving as control. Group 1 hearts (our control group) received glucose potassium cardioplegic solution (G. K. K; 40mEq/L) Group 2 received nicardipine 0.25mg/L in addition to the standard G. K. solution (G. P. K. 0.25). In the third group, the nicardipine dosage was increased to 0.5mg/L (Group 3; G. P. K. 0.5).

Our results indicate that the addition of perdipine to cardioplegic solution provides significant protection against myocardial injury during ischemic arrest.

The effect of perdipine (nicardipine) on hemodynamics was greatest in Group 2, although, Group 3 also demonstrated significant improvement with regard to several parameters.

There were statistically significant differences between the control group (Group 1) and the experimental groups (2 and 3) with respect to Coronary Flow (CF p<0.01), Cardiac Output (CO p<0.05), and the time required to achieve resuscitation (p<0.01). Additionally, Groups 1 and 2 differed significantly with respect to Peak Systolic Pressure (PSP p<0.05) and Pressure Rate Product (PRP p<0.05). There were no significant differences between Groups 2 and 3. CPK levels were assayed in the three groups, with the average level of CPK in the coronary effluent increasing in the following order: Group 3< 2< 1, with the difference in levels being statistically significant between Groups 1 and 3 (p<0.05). Histological damage to the myocardium was mild; however, significant differences in the appearance of contraction bands in the walls of the septum and left ventricle were noted between the control group (1) versus the experimental groups (2 and 3) with p<0.05.
There were no significant differences in the appearance of the coronary arteries in histologic findings, though there was a significant decrease in CF following resuscitation from ischemic arrest in Group 1. It is conceivable that coronary vasospasm may cause lower CF with resultant ischemia and that Ca antagonist combined with cardioplegic solution may diminish post resuscitation vasospasm, thus diminishing ischemic injury. In conclusion, an addition of perdipine (nicardipine) to cardioplegic solution may protect the myocardium from ischemic damage.

INTRODUCTION

In cardiac surgery, in addition to recent advances in surgical repair of complex congenital anomalies, and multiple valvular diseases, as well as cardiac transplantation, there are additional indications for surgery in patients over age 60 which require prolonged aortic clamp time and are therefore at high risk for intraoperative ischemia. There have been many innovations aimed at diminishing the damage sustained by the myocardium during the period of aortic clamping, these include topical cooling \(^{(1)}\) and modifications in the cardioplegic solutions. Topical cooling was first introduced by Shumway \(^{(2)}\) in 1959 and Cardioplegia using hyperkalemic solution was devised by Merlose \(^{(3)}\) in 1955. Cardioplegic solutions are generally composed of 20 to 40mEq/L of potassium in the U.S.A., while Mg is utilized in place of potassium in Europe. The efficacy of cardioplegia in protecting the myocardium is now clear, and greater than three hours duration of aortic clamp has become clinically feasible and safe. Since Zimmerman \(^{(4)}\) (1967) theorized a phenomenon of calcium paradox, attention has been paid to the possibility that the use of Ca antagonists may protect the myocardium from ischemic damage. In this regard, favorable effects of nifedipine were reported by Magovern \(^{(5)}\) and Clark \(^{(6)}\), and the efficacy and optimal concentrations of verapamil, nifedipine, and diltiazem were demonstrated by perfusion studies on rat heart preparations \(^{(7)}\). The current study was undertaken to provide a thorough evaluation of a new effectiveness of a Ca-channel blocker in protecting the myocardium from ischemic damage by utilizing perdipine (nicardipine; Fig. 1) at two concentrations.

![Molecular structure of Nicardipine (YC-93)](image-url)
MATERIAL

Twenty seven male rats (225–330g body weight) of the Wister Strain were used in this study.

ISOLATED WORKING HEART APPARATUS (I.W.H.A.)\textsuperscript{10–14} Fig. 2, Fig. 3, as first devised by NEELY in 1967, is composed of both a retrograde LANGENDORFF aortic perfusion system, and an antegrade working heart made perfusion system. The apparatus was maintained at 37°C using the thermostat (Modular Bath Linear Cool, CR101, N.R.K.). LANGENDORFF reservoir was connected to a perfusate oxygenator and maintained at 75cm above the heart using a constant head device. While the heart is in working mode, perfusate circulates, passing from oxygenating chamber, through filter (24 N.R.K.) to the left atrium by use of a peristaltic pump (HR FLOW INDUCER–WATSON, MARLOW) and returns to the oxygenating chamber via the left ventricle, aortic

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Perfusion apparatus and experimental model in this study}
\end{figure}
pressure chamber and aortic bubble trap in sequential order. The apparatus is equipped with side arm one way valve (ball valve), enabling it to increase coronary flow during all phases of the cardiac cycle. Pressure and heart rate were monitored by pressure chamber transducer (GOULD POLYGRAPH 141-6, SAN-EI). Effluent from heart chamber and aortic bubble trap were collected at various intervals and used to determine coronary flow and aortic flow. PEAK SYSTOLIC PRESSURE (PSP mmHg), HEART RATE (HR/min), PRESSURE RATE PRODUCT (PRP), CORONARY FLOW (CF ml/min), AORTIC FLOW (AF ml/min), CARDIAC OUTPUT (CO ml/min), and STROKE VOLUME (SV 10⁻¹ ml/min) were measured from the above data.

MODIFIED KREBS–HENSELEIT BICARBONATE BUFFER (K.H.B.) (Table 1) was used in this experiment as the perfusate, solute concentrations pH and gas analysis are listed in Table 1.

CARDIOPLEGIC SOLUTIONS;
The three cardioplegic solutions used in this study (Table 2) consist of: Group 1–(G.K)– containing 5% glucose 500ml, KCl (40mEq in 20ml) 10ml, NaHCO₃ (7w/v%) 5ml; Group 2–(G.P.K. 0.25)– with 0.25mg/L of nicardipine (perdipine, YAMANOUCHI Co.) added to G.K. solution; and Group 3–(G.P.K. 0.5)– with 0.5mg/L of nicardipine added to G.K.
Table 1. Composition of Modified-Krebs-Henseleit Bicarbonate Buffer used in this study as perfusate showing pH and gas analysis.

<p>| | | | |</p>
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<tbody>
<tr>
<td>1.</td>
<td>Glucose</td>
<td>11.0mM</td>
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<tr>
<td>2.</td>
<td>NaCl</td>
<td>118.0</td>
<td>PH</td>
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<tr>
<td>3.</td>
<td>KCl</td>
<td>4.7</td>
<td>PCo₂</td>
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<tr>
<td>4.</td>
<td>CaCl₂-2H₂O</td>
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<td>Po₂</td>
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<tr>
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<td>MgSO₄-7H₂O</td>
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<td>HCO⁻</td>
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<td>6.</td>
<td>KH₂PO₄</td>
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<td>BE</td>
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<td>7.</td>
<td>EDTA</td>
<td>0.5</td>
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<tr>
<td>8.</td>
<td>NaHCO₃</td>
<td>2.5</td>
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<td>9.</td>
<td>Distilled H₂O</td>
<td>1000ml</td>
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Table 2. The composition of the three cardioplegic solutions used in this experiment.

<table>
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<th>Group 2</th>
<th>Group 3</th>
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<tr>
<td>5%GLUCOSE</td>
<td>500ml</td>
<td>500ml</td>
<td>500ml</td>
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<tr>
<td>HEPARIN</td>
<td>1ml</td>
<td>1ml</td>
<td>1ml</td>
</tr>
<tr>
<td>KCl(20ml,40mEq)</td>
<td>10ml</td>
<td>10ml</td>
<td>10ml</td>
</tr>
<tr>
<td>NaHCO₃(7%/v%)</td>
<td>5ml</td>
<td>5ml</td>
<td>5ml</td>
</tr>
<tr>
<td>Nicardipine (Perdipine)</td>
<td>0.125mg</td>
<td>Nicardipine (Perdipine)</td>
<td>0.25mg</td>
</tr>
</tbody>
</table>

METHODS

Wister strain rats were anesthetized with ether and a vertical midline incision was made to expose the abdominal and thoracic cavities. Next, 1 ml of heparin was administered via the femoral vein or vena cava, and the heart was removed by making a single cut through the left atrium, severing the pulmonary veins and other vessels arising from the heart, which was placed into cold K.H.B.-solution causing arrest within seconds. The aorta was then cannulated followed by five minutes of LANGENDORFF perfusion (with resumption of spontaneous cardiac contraction within 10 seconds) during which time a left atrial cannula was introduced through the pulmonary vein. Thereafter, the retrograde LANGENDORFF perfusion system was clamped and the working heart preparation was run continuously for 10 minutes at a preload of 10cmH₂O and an afterload of 75cmH₂O. At this time, hemodynamic parameters such as PSP, HR, CF, AF were along with a one minutes collection of effluent for CPK analysis in order to establish baseline values. Next, 5ml of cardioplegic solutions, which were previously described for Groups 1, 2, 3 were administered over a one minute period by gravitational flow from a height of 60cm above the heart via the retrograde LANGENDORFF perfusion system, with cardiac arrest occurring within seconds of initiation of flow. The retrograde perfusion
Table 3. Hemodynamic parameters and heart weight in the three groups immediately prior to exposure to cardioplegic solution P.S.P. (Peak Systolic Pressure), H.R. (Heart Rate), C.F. (coronary Flow), A. F. (Aortic Flow), C.O. (Cardiac Output).

<table>
<thead>
<tr>
<th>Group 1: GK n=9</th>
<th>WEIGHT HEART g</th>
<th>P.S.P. mmHg</th>
<th>H.R. ml/min</th>
<th>C.F. ml/min</th>
<th>A.F. ml/min</th>
<th>C.O. ml/min</th>
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</thead>
<tbody>
<tr>
<td>288±32 (1.39±0.17)</td>
<td>99.1±7.3</td>
<td>189±27</td>
<td>9.9±1.0</td>
<td>24.0±5.7</td>
<td>33.9±6.2</td>
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<table>
<thead>
<tr>
<th>Group 2: GPK0.25 n=9</th>
<th>WEIGHT HEART g</th>
<th>P.S.P. mmHg</th>
<th>H.R. ml/min</th>
<th>C.F. ml/min</th>
<th>A.F. ml/min</th>
<th>C.O. ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>272±34 (1.38±0.17)</td>
<td>93.4±13.5</td>
<td>201±45</td>
<td>10.2±1.4</td>
<td>23.1±6.7</td>
<td>33.3±7.2</td>
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<table>
<thead>
<tr>
<th>Group 3: GPK0.5 n=9</th>
<th>WEIGHT HEART g</th>
<th>P.S.P. mmHg</th>
<th>H.R. ml/min</th>
<th>C.F. ml/min</th>
<th>A.F. ml/min</th>
<th>C.O. ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>296±28 (1.44±0.11)</td>
<td>91.0±8.3</td>
<td>202±15</td>
<td>11.0±1.3</td>
<td>22.9±3.0</td>
<td>34.9±3.9</td>
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</tbody>
</table>

Fig. 4. Experimental Time Course in this study
Pictures. The appearance of contraction bands in the wall of septum and left ventricle.
1) Grade 1.; absence of contraction bands.
2) Grade 2.; scattered contraction bands.
3) Grade 3.; extensive presence of contraction bands.
was clamped and the heart maintained in ischemic arrest for to 30 minutes, while in the heart chamber with perfusate circulating at 37°C.

Next the heart was resuscitated by the LANGENDORFF perfusion system and resumed beating within 90 seconds. The retrograde perfusion was continued for a total of ten minutes to washout all cardioplegic residua. At the conclusion of this ten minute period the retrograde LANGENDORFF system was clamped and the working heart preparation resumed. At set time intervals of 5, 10, 15, 20, 30, 40min, the hemodynamic parameters stated above were once again measured (Fig. 4). CPK levels in coronary effluent were measured with use of Clearnizer (Nihondensi, Co.) at three times during the experiment; 1) just prior to induction of ischemic arrest with exposure to cardioplegic solution, (as previously described) 2) at the time of resuscitation with LANGENDORFF preparation, the effluent was pooled over this entire ten minute period and expressed as CPK total, and 3) after twenty minutes of working heart preparation following ischemic arrest, a one minute collection of effluent was obtained and the CPK level determined. Through the experiment, coronary effluent was excluded from recirculation. At the conclusion of the experiment, each heart was weighed by METTLER AE 100 ballance, fixed in 10% formalin, and transversely sectioned so as to include the interventricular septum and the left ventricular wall. Each section was stained with hematoxylin and eosin in addition to the use of AZAN MARORY, PTHA and WEIGERT stain methods. Damage to the heart muscles was expressed by an appearance of contraction bands which were graded as follows; Grade 1-absence of contraction bands, Grade 2-scattered contraction bands and Grade 3-extensive presence of contraction bands as shown in Pictures 1, 2, and 3.

Hemodynamic data were expressed as mean values ± S.D. and as percent of controls. F analysis and Student t-test were used for statistical analysis. P values less than 0.05 were considered to be significant. Statistical significance of CPK values was estimated by MANN-WHITNEY U-test (non-parametric method) with P values of less than 0.05 and the significance of the different grades of structural alteration of the myocardium was estimated by test of contingency table with P-values of less than 0.05.

RESULTS

Hemodynamic changes during experiments in Group 1, 2, and 3 are represented in Fig. 5 a), b), and c) respectively.

PRE-ARREST 10 MIN OF WORKING HEART PREPARATION; During 10 min of the working heart mode hemodynamic parameters of PSP, HR, CF, AF and CO were measured, serving as baseline value for the experiment (see Table 3). The ratio of CF to CO in this phase of the experiment was approximately 1:3.3, which was consistent with the NEELY89 report.

RESUSCITATION TIME; All hearts used in this study were resuscitated within the first 90 seconds of a 10 minute LANGENDORFF perfusion after 30 min of ischemic
Fig. 5. Hemodynamic changes in the three different time courses among Group 1 (G.K') a); Group 2 (G.P.K., 0.25) b); Group 3 (G.P.K., 0.5) c).

arrest. In Groups 1 and 3, one out of the nine isolated hearts in which ischemic cardiac arrest was induced, returned to a normal rhythm after an intervening episode of ventricular fibrillation for 90 and 45 sec, respectively. The remaining hearts began to beat spontaneously without intervening VF. The required resuscitation times during the 10 min LANGENDORFF perfusion in Group 1 was statistically longer than those in

<table>
<thead>
<tr>
<th>Group 1</th>
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<th>Group 3</th>
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<tbody>
<tr>
<td>G.K</td>
<td>GPKO.25</td>
<td>GPKO.5</td>
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<tr>
<td>n = 9</td>
<td>n = 9</td>
<td>n = 9</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>60</td>
<td>90</td>
</tr>
<tr>
<td>○; heart resumed spontaneous contraction after of asystole</td>
<td>○; heart resumed beating after period of ventricular fibrillation</td>
<td>○; heart resumed spontaneous contraction after of asystole</td>
</tr>
<tr>
<td>**; statistically significant p&lt;0.01</td>
<td>**; statistically significant p&lt;0.01</td>
<td>**; statistically significant p&lt;0.01</td>
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</table>
Groups 2 and 3 \( (p<0.01) \) as indicated in Table 4.

**POST ARREST 10 MINUTES RETROGRADE LANGENDORFF PREPARATION**; A comparison of coronary effluent volume to heart weight was made among the three groups in a 10 minutes LANGENDORFF preparation, as this is known to be directly related to the degree of coronary artery distension, there was no statistical significance as shown in Table 5.

**PEAK SYSTOLIC PRESSURE (PSP)**; All three Groups approached approximate baseline values, demonstrating variable success, with \( 1<3<2 \), the difference between Groups 1 and 2 being statistically significant \( (p<0.05) \) Table 6 and Fig. 6.

**HEART RATE (HR)**; Heart rate inhibitory effects of nicardipine were not apparent at concentration of 0.25 or 0.5mg/L. All groups returned to baseline rates with no significant intergroup differences. (Fig. 6 b).

**PRESSURE RATE PRODUCT (PRP)**; There was relative preservation of the PRP in the groups exposed to nicardipine Groups 2 and 3 as compared to the control, with significant difference \( (p<0.05) \) between groups 1 and 2 after twenty and forty minutes of the working mode. (Fig. 6 c).

**CORONARY FLOW (CF)**; Values were determined beginning at 5 min following commencement of the working heart mode \( (i.e.; 15 \text{ minutes post resuscitation from ischemic arrest}) \). No statistical differences were noted between the three groups at five minutes (Fig. 7 a). A remarkable decrease in coronary flow, however, was noted in Group 1 by 10 min working heart mode (or 20 min post resuscitation), with a decrease

<table>
<thead>
<tr>
<th>Group</th>
<th>GPKO</th>
<th>n=9</th>
<th>50</th>
<th>100 ml/gr</th>
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<tbody>
<tr>
<td>Group 1</td>
<td>70.5±10.7</td>
<td></td>
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<tr>
<td>Group 2</td>
<td>77.7±10.1</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>78.7±12.1</td>
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</table>

*Table 5.* Index of coronary artery distension as measured by the ratio of perfusate volume passing through the coronary arteries versus heart weight in a 10 minutes Langendorff perfusion study. (ml/g)
Table 6. Hemodynamic changes with time among the three groups. (expressed as a percent of their preischemic values ± S.D.,

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<tr>
<th></th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>20 min</th>
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<tr>
<td>G K</td>
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<tr>
<td><em>n=9</em></td>
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<tr>
<td>P.S.P.</td>
<td>83.2±10.4</td>
<td>82.7±8.2</td>
<td>85.9±8.8</td>
<td>87.5±8.3</td>
<td>89.1±9.1</td>
<td>89.4±9.9</td>
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<tr>
<td>H. R.</td>
<td>95.±22.0</td>
<td>97.5±24.2</td>
<td>100±20.5</td>
<td>102±18.7</td>
<td>106±15.5</td>
<td>107±15.2</td>
</tr>
<tr>
<td>P.R.P.</td>
<td>77.±16.3</td>
<td>80.9±19.3</td>
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<td>86.±11.9</td>
<td>93.±10.8</td>
<td>94.±9.9</td>
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<tr>
<td>C.F.</td>
<td>99.±10.5</td>
<td>81.±20.4</td>
<td>76.±16.8</td>
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<td>A. F.</td>
<td>44.±29.6</td>
<td>55.±24.8</td>
<td>73.±30.7</td>
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<td>103±13.6</td>
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<td>C. O.</td>
<td>61.±21.3</td>
<td>64.±17.4</td>
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<td>80.±11.9</td>
<td>87.±10.2</td>
<td>92.±12.3</td>
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<td>S. V.</td>
<td>64.±19.6</td>
<td>67.±17.4</td>
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<td>82.±16.9</td>
<td>84.±16.5</td>
<td>88.±15.6</td>
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<td><strong>Group 2</strong></td>
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</tr>
<tr>
<td>H. R.</td>
<td>93.±23.1</td>
<td>97.±23.0</td>
<td>100±17.4</td>
<td>103±11.6</td>
<td>104±10.6</td>
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<td>P.R.P.</td>
<td>81.±20.8</td>
<td>87.±22.0</td>
<td>92.±16.2</td>
<td>99.±9.5</td>
<td>*102±10.6</td>
<td>105±8.2 *</td>
</tr>
<tr>
<td>C. F.</td>
<td>102±13.0</td>
<td>100±11.2</td>
<td>99.±12.0</td>
<td>100±8.9</td>
<td><strong>99.±9.2</strong></td>
<td>101±11.3 **</td>
</tr>
<tr>
<td>A. F.</td>
<td>50.±28.8</td>
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<td>74±24.5</td>
<td>91.±15.3</td>
<td>103±11.9</td>
<td>107±14.1</td>
</tr>
<tr>
<td>C. O.</td>
<td>68.±17.7</td>
<td>74.±17.8</td>
<td>82.±16.5</td>
<td>93.9±15.3</td>
<td>*102±8.2 **</td>
<td>105±10.2 **</td>
</tr>
<tr>
<td>S. V.</td>
<td>74.±16.7</td>
<td>78.±13.7</td>
<td>83.±13.9</td>
<td>92.±15.7</td>
<td>98.±14.3</td>
<td>99.7±13.8</td>
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<td>P.S.P.</td>
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<td>H. R.</td>
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<td>107±17.8</td>
<td>108±16.6</td>
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<tr>
<td>P.R.P.</td>
<td>82.±7.7</td>
<td>86.±7.5</td>
<td>92.±8.1</td>
<td>95.0±7.3</td>
<td>101±9.1</td>
<td>104±9.2</td>
</tr>
<tr>
<td>C. F.</td>
<td>97.±5.9</td>
<td>98.±8.0</td>
<td>97.±8.8</td>
<td><strong>97.±5.2</strong></td>
<td><strong>98.±8.7</strong></td>
<td><strong>98.3±8.9</strong></td>
</tr>
<tr>
<td>A. F.</td>
<td>44.±16.6</td>
<td>61.±17.3</td>
<td>72.±14.6</td>
<td>81.±10.1</td>
<td>97.5±8.6</td>
<td>104.±6.2</td>
</tr>
<tr>
<td>C. O.</td>
<td>62.±13.8</td>
<td>74.±12.3</td>
<td>81.±11.0</td>
<td>86.±7.9</td>
<td>97.±6.5 **</td>
<td>102±6.3</td>
</tr>
<tr>
<td>S. V.</td>
<td>65.±14.4</td>
<td>75.±17.9</td>
<td>76.±10.7</td>
<td>83.±6.5</td>
<td>93.±11.1</td>
<td>95.±13.0</td>
</tr>
</tbody>
</table>

**; statistically significant p<0.01, *; p<0.05

Fig. 6. Changes in hemodynamic parameter of PEAK SYSTOLIC PRESSURE a) HEART RATE b), PRESSURE RATE PRODUCT c) after 30 minutes of ischemic arrest, (expressed as a percent of preischemic values ± S.D., ○——○; G. k. Group 1, •—•; G. P. K. 0.25 Group 2, ▲—▲; G. P. K. 0.5 Group 3, *; statistical significance p<0.05, **; statistical significance p<0.01)
in flow of 74%. On the other hand, the values in Groups 2 and 3 remained close to the initial levels. This decrease in Group 1 was maintained throughout the experiment as measurements at 10, 15, 20, 30 and 40 minutes of working mode revealed. The difference in flow at all of these time points was statistically significant ($p<0.01$), as compared with Groups 2 and 3.

**AORTIC FLOW (AF);** This increased among the three groups (as compared to baseline) at an interval of 30 to 40 min after commencement of working heart mode, followed by a constant flow. There was no statistical difference among the three groups (Fig. 7b).

**CARDIAC OUTPUT (CO);** This also rapidly increased after the start of perfusion. Statistical differences between Groups 1 and 2 were noted as ($p<0.05$) at 20 and 40 min after resumption of the working heart mode ($p<0.01$) at the 30 min time point. A difference between Groups 1 and 3 of less than 0.05 was observed after 30 min of beating as indicated in Fig. 7c.
STROKE VOLUME (SV); The values in Groups 2 and 3 increased more than those in Group 1, however, there was no statistical difference among the three groups as illustrated in Fig. 7d.

ENZYME LEAKAGE (CPK); These values were expressed as a) the ratio of total CPK during a 10 min Langendorff perfusion to heart weight, b) the ratio of CPK which leaked 10 min to total CPK which leaked out during 1 min of the control (CPK control) and c) the ratio of CPK for 20 min of reworking perfusion to CPK for the control. These values varied with a wide range, but mean values decreased in the order; Group 1 > 2 > 3. MANN-WHITNEY U-test was used for statistical analysis, and significant difference (p < 0.05) existed between Groups 1 and 3 Fig. 7a, b, c.

HISTOLOGIC STRUCTURAL ALTERATIONS; Isolated hearts were examined histologically at completion of the experiments. Histology revealed marked edema within intercellular connections.

Table 7. C.P.K. leakage compared with preischemic values.

<table>
<thead>
<tr>
<th>Group</th>
<th>n=9</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*; statistical significance p < 0.05.

Table 8. Incidence of histologic finding of contraction band on the myocardium according to its grading

<table>
<thead>
<tr>
<th>grade of contraction band appearance</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 n=9</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Group 2 n=9</td>
<td>2</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Group 3 n=9</td>
<td>7</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

statistically significant p < 0.05. Groups 1 and 2, Groups 1 and 3, data were analyzed by multiple sample chi-square test.
Damage to the heart muscle was expressed by evaluation of the extent of appearance of contraction bands as indicated in Table 8. Group 1 had the highest incidence with significant differences (p<0.05) between Groups 1 and 2, and 1 and 3.

DISCUSSION

Perfusion of the isolated heart was developed by LANGENDORFF in 1895, in which the coronary arteries were perfused via retrograde flow into the aorta. This technique was improved by NEELY in 1967 by addition of a "working mode" in which flow was antegrade through the heart via canulation of the pulmonary vein. This is not considered a physiologic state, as no hemoglobin is utilized and oxygen circulates in the dissolved form. Additionally, both pre- and after-load were fixed. Nevertheless, several features made this technique, a valuable for instance it allows for direct measurements of coronary and aortic flows in addition to analysis of metabolites released from the heart muscle, and the system is both simple and readily reproducible the reproducability is supported by our finding that the CF to CO ratio was 1:3.3, which is consistent with data reported by NEELY using the same system.

A possibility of reperfusion injury on the myocardium was proposed by ZIMMERMAN in 1967. He proposed that Ca ion influx across the subsequent to cell membrane during perfusion with a calcium containing buffer, after calcium free buffer perfusion, plays an important role in damage to the mitochondria preceding cell death. The myocardial protective effects of a Ca antagonist on preventing this "calcium paradox" were supported by clinical data of MAGOVERN and CLARK. The addition of nifedipine to cardioplegic solutions demonstrated to have certain advantages over traditional cardioplegic solutions in enhancing myocardial protection. In this regard, the effect of verapamil and nicardipine were recognized by SHIKANO (1983) and YAMAMOTO (1983), who among other things, determined the optimal concentrations of calcium antagonists to be, 1.0μmol/L of verapamil, 0.075μmol/L of nifedipine and 0.50μmol/L ditiazem. These specificity concentrations significantly increased AF by 20, 60 and 50%, respectively, as well as reducing CK level by 30, 50 and 40% in LANGENDORFF resuscitating effluent that study utilized St. THOMAS cardioplegic solution (which contained significant levels of Ca and Mg in addition to solution) and other experimental model. This study demonstrated 16.3% increase in CO with the addition of nicardipine, with most favorable results occuring in the group which received 0.25mg/L of the drug.

CF rapidly decreased in Group 1 by 10 min into the working heart mode. It is generally believed that reduction of CF after the heart has resumed beating is due to vasospasm of the coronary arteries. VAN NUETEN (1980) reported that the use of lidoflazin (Ca antagonist) effectively inhibits coronary vasospasm. BURTON also noted that verapamil acts to completely relieve coronary vasospasm induced by KCl, as well as inhibiting coronary contraction induced by 5HT and angiotensin. NAKAYAMA reported the fact of transmembrane Ca ion flux into coronary artery smooth muscle cells previously
depolarized by potassium based cardioplegia. That coronary artery spasm is associated with Ca flux causes coronary vasospasm. KONDO et al.\textsuperscript{21}, is further supported by demonstrated inhibition of spasm by nifedipine, diltiazem and verapamil. And LAZZETTIR et al\textsuperscript{22}, noted that nicardipine is more effective than papaverin in inhibiting potassium induced coronary artery contraction. It follows from these observations that the addition of a Ca antagonist to potassium based cardioplegic solution may maintain coronary flow by inhibiting coronary spasm.

A furthermore, damage to the myocardium as reflected by CPK leakage was significantly diminished in the group receiving the higher concentration of Ca antagonist.

Structural alterations of the myocardium were indicated by the presence of contraction bands. As cited by GONOTE, C. E.\textsuperscript{23}, contraction bands are observed after reoxygenation of myocardium following hypoxic injury as well as in the setting of the "Ca paradox" which is observed when an isolated heart is perfused by a calcium containing buffer post perfusion with a Ca-free buffer. As it is hypothesized that the damage is secondarily increased Ca flux with resultant vasospasm, Ca antagonist may play a role in elimination of the calcium paradox.

In this study, we demonstrated that coronary vasospasm may not occur until 15 minutes after reperfusion of ischemic hearts. Thus, a phenomenon of post ischemic distension of the coronary artery may continue to be potentiated for sometime following resuscitation, and that coronary vasospasm induced by transmembrane Ca ion flux into coronary smooth muscle cells may be delayed 15 minutes or more. Our finding therefore in part contradict those of YAMAMOTO et al. who indicated that post ischemic verapamil administration by LANGENDORFF preparation did not remarkably alter hemodynamics or CK leakage in isolated heart preparations. Based upon our findings, we recommend that for enhancing myocardial protection, during potassium-based cardioplegia the addition of a Ca antagonist is indicated.

Further studies of nicardipine and other Ca antagonist in conjunction with experimental and clinical investigations are warranted. We believe that Ca antagonists may offer an important new dimension to myocardial protection in settings requiring cardioplegia.

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